Intact Protein Liquid Chromatography Mass Spectrometry for Bacteria Strain Differentiation and Bacterial Toxin Detection

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Novel Aspect:
Intact protein liquid chromatography mass spectrometry is used to differentiate bacterial serovars and strains using protein profiles.

Introduction:
Bacterial species can be easily differentiated by many genetic and chemical methods. More precise tools are necessary, however, to differentiate bacteria at the serovar, strain and substrain levels. We have used liquid chromatography mass spectrometry to characterize the measurable expressed proteins from bacteria; this method has previously been used to distinguish groups of bacteria within a species, as in the case of thermostable and non-termostable strains of *Enterobacter sakazakii*. Here we extend this method to show that serovar and strain-level differentiation of *Salmonella enterica* and *E coli* can be accomplished. Moreover, the approach can readily determine the induction of bacterial toxins, such as shigatoxin, in pathogenic *E. coli*.

Methods:
E. coli O157:H7. *E. coli* K12 , and 72 strains of *Salmonella enterica* reference set A (SAR A) were analyzed. Bacteria grown to stationary phase were repetitively extracted at 30,000 psi with a 50:45:5 mixture of acetonitrile:water:formic acid using a Barocycler. Extracts were analyzed by LC/MS on an Agilent 1100 LC with a Waters QTOF Premier Mass Spectrometer. Proteins are separated on a Prosphere HR nonporous PSDVB column (4 micron particles) with a gradient of water/acetontirile/5% formic acid at 200 µl/min. LC/MS runs are analyzed using ProTrawler 6 data analysis package (BioAnalyte, Inc), which performs noise reduction, maximum entropy deconvolution and reconstruction of the chromatogram to yield a list of mass, retention times and intensities.

Preliminary Results:
LC/MS measurements of bacterial extracts were used to generate protein profiles for the 72 strains of SAR A, as well as various strains of *E. coli* O157:H7. Replicate sets of bacterial cultures/extracts were analyzed to assess the precision of the protein profile measurements. 200-600 proteins are typically observed in the protein profiles. Coefficients of variation differ with the specific protein biomarker, ranging from 5-40%; CVs do not directly correlate with protein abundance and some low abundance proteins have relatively low CVs. Protein profiles easily differentiate species, and can also differentiate serovars and strains, as shown by cluster analysis and principal components analysis of the data. Comparison of dendrograms from protein profiles with phylogenetic trees based on multilocus sequence typing (MLST) shows that the protein profiles can differentiate strains that cannot be differentiated by MLST. Further analysis of the protein profiles can be used to identify biomarkers that specifically characterize serovars and strains. The methodology has also been used to distinguish sequenced strains of *E. coli* O157:H7 (Sakai and EDL933), and appears to differentiate different strains of *E. coli* O157:H7 isolated from food borne outbreaks. The method can also be used to identify changes induced in bacteria by external conditions. For example, addition of ciprofloxacin to cultures of EDL933 induces phage production, which in turn results in lysis of the bacterium and the release of shigatoxins to the surrounding medium. The approach described here can be used to readily monitor the appearance of the shigatoxins.